

National Program 301 • Plant Genetic Resources, Genomics, and Genetic Improvement FY 2013 Annual Report

The USDA-Agricultural Research Service's (ARS) National Program (NP) 301, Plant Genetic Resources, Genomics, and Genetic Improvement, supports research that maintains, protects, enhances, and expands the United States genetic resource and information base, and increases knowledge of the structure and function of plant genes, genomes, and biological and molecular processes. Through innovative research tools and approaches, this National Program manages, integrates, and delivers vast amounts of genetic, molecular, biological, and phenotypic information to a diverse global clientele. The ultimate goals for the preceding efforts are to improve the production efficiency, yield, sustainability, resilience, health, product quality, and value of U.S. crops. This National Program addresses the need to:

- Ensure the long-term safety and integrity of our agriculturally valuable genetic resource collections and associated information;
- Develop novel approaches for analyzing complex traits, identifying favorable genes underlying those traits, and deploying them in breeding stocks;
- Exploit new technologies that enhance traditional methods of genetic improvement;
- Genetically improve a broad spectrum of major, specialty, and new crops;
- Increase our knowledge of the structure and function of crop genomes and of plant biological and molecular processes; and
- Acquire, analyze, and deliver genetic and information resources to research communities and to the public.

Genetic resources are the foundation of humanity's agricultural future. ARS genebanks contain sources of resistance, tolerance, and adaptation to biotic and abiotic stresses and new genes to improve the quantity, quality, and production efficiency of our food, feed, bioenergy, fiber, and ornamental crops. To ensure those genes are available for research and breeding, ARS must continue to strategically acquire and conserve germplasm that contains them; develop new screening methods for identifying favorable traits; ensure that germplasm is distributed where and when it is needed; and safeguard these collections for future generations.

ARS has taken a leadership role in developing and curating crop genomic and phenotypic databases, which are expanding now and will continue to do so in the future. New tools are needed to efficiently capture phenotypic data and extract useful information, via innovative data-mining strategies, from the ever-increasing flow of data into these databases. Methods to interconnect databases containing diverse types of information are needed to more efficiently and effectively identify important properties of genes and genomes and apply that knowledge to crop improvement. In addition, these interconnected databases will enable researchers to better associate specific genes with agriculturally-important traits and build on genetic advances in one crop to speed genetic gain in others.

For major, specialty, and new crops, ARS, in close cooperation with diverse public and private sector collaborators, will improve and broaden the genetic base of U.S. crops to reduce genetic vulnerability. NP 301 scientists will devise and apply new technologies to develop superior new crop varieties and

enhanced germplasm and will accelerate the deployment of high-value traits into breeding populations. New genetic sources of key crop traits will be identified and incorporated into crop breeding lines and gene pools. New breeding theories and strategies that exploit the vast amount of available genotypic data will be developed to effectively capture the intrinsic genetic potential in germplasm. To do so, innovative tools will be developed for dissecting the architecture of complex crop traits. These tools will be applied to mapping and determining the function of underlying genes so that variation in them, and in linked genomic sequences, can be exploited as more precise genetic markers. This knowledge will also enable molecular and genetic variability to be associated with specific phenotypes to enhance the effectiveness of genomic selection, thereby accelerating and strengthening progress in crop genetic improvement.

Plant breeding has a long history of improving crops through phenotypic or marker-assisted selection, usually in the absence of information regarding underlying genes or gene networks that control key traits, or that control how plants interact with their environment. Understanding the key biochemical pathways and the underlying controls for plant growth, development, and metabolism has been elusive. However, recent advances in plant molecular biology, genetic engineering, and physiology could create opportunities for improvement of crops by more precisely and rapidly manipulating the existing variability in plant properties, or by creating new variability when warranted. Knowledge of how to precisely modify biological mechanisms will contribute not only to advances in fundamental plant biology, but will also help identify potential unintended consequences of that manipulation.

In addition to conducting research, ARS and its university cooperators will continue to mentor and train the next generation of crop breeders, geneticists, bioinformaticists, genome researchers, and genetic and genomic resource and information managers. Developing the next cohort of researchers is particularly important, considering the projected strong demands for these scientific personnel.

This National Program is composed of three Research Components (see below) and their constituent Problem Statements. Notably, these categories do not act as barriers or “stovepipes” that impede research across the National Program. Rather, many of the individual research projects and resources contribute to the goals of several NP 301 Research Components, or even several National Programs:

- Crop Genetic Improvement (plant breeding, genetic enhancement, molecular marker and trait analyses, nucleotide sequencing, and genetic mapping).
- Crop Genetic and Genomic Resources and Information Management (conserving a broad spectrum of genetic resources and facilitating their use; maintaining and delivering genetic resource, genomic, and genetic data via databases and information management systems; and devising new analytical tools that compile, dissect, interrelate, manage, and visualize the information contained in the large datasets).
- Crop Biological and Molecular Processes (fundamental – often high-risk – research to generate an enhanced knowledge base and devise superior research tools that ultimately contribute to greater crop productivity and efficiency; better product quality and safety; improved protection against pests and diseases; enhanced tolerance to abiotic stress; and sustainable practices that maintain or enhance environmental quality).

The following section contains selected NP 301 accomplishments, presented according to the relevant NP 301 Research Components. These are not all of the NP 301 accomplishments for FY 2013, but rather are selected based on impact and level of contribution to meeting the National Program goals.

Component 1 – Crop Genetic Improvement

'Sunpreme' raisins dry themselves. Eliminating the processing step of tray-drying in the field would help maintain the quality of raisins and reduce the need for costly additional post-harvest processing. ARS researchers in Parlier, California, have developed 'Sunpreme,' a new raisin grape that dries naturally on the vine without the grapes requiring cutting and drying in trays. Sunpreme is particularly well-suited for mechanical harvesting, thereby significantly reducing production costs. Unlike Thompson Seedless, the major grape variety used for tray-dried raisin production, Sunpreme can be spur-pruned, further reducing grower costs. (NP301, C1, PS 1A, PM 1.3.1; Project #5302-21220-005-00D).

Discovery of genes for drought tolerance in common bean. Drought strongly reduces the yields of dry beans in the Northern Plains (North Dakota and Minnesota), Great Lakes (Michigan), and other regions which rely primarily on rainfall for crop growth. Consequently, dry beans with tolerance to drought are critical for those regions, and for adapting this globally important crop to climate change. ARS researchers in Prosser, Washington, and their university colleagues identified two major genes (also known as quantitative trait loci or QTL) that strongly governed drought tolerance in dry beans. The two genes showed positive effects when the dry beans were cultivated in multiple drought-stress environments. This research will enhance capacity for marker-assisted breeding to accelerate development of drought-tolerant beans by seed companies and public-sector breeders in the United States and worldwide. (NP 301; C 1; PS 1B; PM 1.3.1; Project #5354-21220-016-00D).

40,000 new genetic markers for evaluating the diversity in the USDA soybean germplasm collection. New genetic tools are needed to accelerate the pace of mining germplasm collections for valuable genes for breeding and research. ARS researchers in Beltsville, Maryland, mapped more than 40,000 single nucleotide polymorphism (SNP) DNA markers to specific chromosomal locations on the soybean genome to facilitate identifying genes that control traits of interest. These extensive data were deposited in SoyBase, the ARS soybean genome database, which is consulted heavily by public and private-sector researchers worldwide. The researchers applied the suite of 40,000 SNP DNA markers to analyze the 18,000 soybean samples in the ARS germplasm collection to identify an optimal core subset that would increase the effectiveness of genebank management. In doing so, they identified more than 4,300 samples that were 99.9 percent genetically identical and could be omitted from further study. With the remaining samples, a core subset of about 1,400 samples was chosen that represented 79 percent of the total genetic diversity of the entire collection. This core subset enables soybean breeders and geneticists to search for key traits from a relatively small and manageable number of samples representing most of the genetic variability of the entire collection. (NP 301, C1, PS 1B; PM 1.3.1; Project #1275-21000-263-00D).

Novel multi-seeded mutants identified that might substantially increase sorghum grain yield. ARS researchers in Lubbock, Texas, have identified novel sorghum mutants that might significantly increase sorghum grain yield. The multi-seeded mutants have more and larger primary and secondary flower branches bearing more types of floral organs that develop into seeds. Compared to current sorghum cultivars, these mutants have triple the number of seeds and double the seed weight. The mutants are being evaluated in the field for their ability to substantially increase sorghum grain yield. (NP301; C1; PS 1A; PM 1.3.1; Project#6208-21000-017-00D).

Wheat gene Mds-1 governs susceptibility to Hessian fly. Hessian fly stunts and kills wheat seedlings and causes adult plants to fall over, or lodge. Wheat resistance genes against Hessian fly are rapidly overcome by new genetic biotypes of the Hessian fly, so new strategies are needed to combat this pest. ARS researchers in Manhattan, Kansas, and their university colleagues found a wheat gene, Mds-1, which confers susceptibility to Hessian fly. When this gene was deactivated experimentally, wheat plants became immune to all biotypes of Hessian fly. Thus, modification of susceptibility genes such as Mds-1 may confer to wheat a potentially broad and durable resistance to Hessian fly and other insects. (NP301; C1; PS 1B; PM 1.3.1; Project#5430-21000-006-00D).

‘Flavorfest’ strawberry cultivar released. Mid-Atlantic strawberry growers face an increasing problem from plant loss and fruit-rot from the current leading strawberry variety because of its susceptibility to the fungal disease, anthracnose. ARS scientists in Beltsville, Maryland, have released the ‘Flavorfest’ strawberry that is resistant to plant and fruit-rot anthracnose, has a similar season to the current leading variety, and delivers up to a two-fold higher yield of large berries with excellent flavor and creamy texture. Mid-Atlantic strawberry crop consultants and extension agents are advising growers to try ‘Flavorfest’ and consider it a replacement for the current leading variety. (NP 301, C1, PS 1A, PM 1.3.1; Project No: 1245-21220-189-00D).

‘Mesa,’ first Russian wheat aphid-resistant winter feed barley variety released. Russian wheat aphid, an invasive pest of small grains, is a major problem for small grain production, requiring repeated applications of the most environmentally detrimental pesticides as a control measure. ARS researchers in Stillwater, Oklahoma, developed a new barley variety, ‘Mesa,’ with high levels of inherent resistance to both the U.S. types of Russian wheat aphid and those found in other parts of the world. With continued soil erosion in the western United States and new demands for a feed alternative to corn, Russian wheat aphid-resistant barley represents an advantageous environmental and economic crop option for High Plains growers. (NP301; C1; PS 1A; PM 1.3.1; Project #6406-21000-011-00D).

High-resolution genetic maps for grapevine. Plant breeders and geneticists place DNA markers on genetic maps to track the location of genes underlying key traits, such as disease resistance and fruit quality. Akin to mile markers on highways, DNA markers reveal the context of the destination (the trait), and more numerous markers result in more precision in reaching the destination. ARS researchers at Geneva, New York, and Ithaca, New York, adapted a new genetic technology that resulted in a 10-fold increase in the number of DNA markers in grapevine, at a cost that is affordable to grape breeders. As a result, traits such as powdery mildew resistance, flower sex, seedlessness, and berry color are being tracked in traditional grape breeding programs with dramatically improved precision. These markers enable breeders to identify and discard undesirable progeny within months of seed germination, saving years of effort and expense in the breeding and evaluation process. (NP 301, C1, PS 1B; PM 1.3.1; Project #1910-21220-004-00D)

Genes from wild soybeans in USDA-ARS soybean collection incorporated into adapted soybean breeding lines for the first time. Genes from the wild soybean had not previously been exploited for increasing soybean yield because of the wild soybean’s undesirable plant growth habit, seed type, and other traits. ARS researchers at Urbana, Illinois, applied marker-assisted plant breeding to develop high yielding soybean lines that yielded as much as the adapted soybean parents but contained much of the genetic diversity from the wild soybeans. Analyses with more than 1,500 DNA markers determined that these lines were genetically unique, with some of the high yielding lines containing more than 30 percent of the genes from the wild soybean source, a much higher

level than the expected 12 percent. These lines are serving as new breeding stocks for expanding the genetic diversity and increasing yield in future U.S. soybean varieties.(NP 301, C1, PS 1B, PM 1.31; Project #3611-21000-023-00D).

Rootstocks that significantly improve citrus fruit production under severe huanglongbing disease pressure. Huanglongbing (citrus greening) disease is widespread in Florida and severely affects health of citrus trees. ARS researchers in Ft. Pierce, Florida, compared tree health and fruit production of sweet orange and other citrus cultivars by grafting them on numerous types of rootstocks in field trials located in regions severely affected by huanglongbing. The research demonstrated significant rootstock differences in fruit production under conditions of severe huanglongbing disease. Some rootstocks enable citrus trees to better tolerate huanglongbing disease and suggest that a disease-tolerant rootstock will be one component of successful citrus production management in the presence of that disease. (NP 301, C1, PS 1A; PM 1.3.1; Project # 6618-21000-013-00D).

Identification of thermo-insensitive lettuce varieties. Lettuce is a cool season crop, and its seeds fail to germinate at temperatures higher than 28° to 32°C because of “thermo-dormancy.” ARS researchers in Salinas, California, screened more than 3,500 lettuce varieties in controlled environments and fields and identified varieties of crisphead, romaine, leaf, butterhead, and wild lettuces with seeds that can germinate at 34°C. These thermo-tolerant varieties could expand lettuce production seasons to warmer regions with lower costs of land, and also reduce the need for “seed priming” which would lower production costs. These findings will help growers choose lettuce varieties for warm environments and help lettuce breeders to improve the crop for adaptation to global warming and climate changes. (NP 301, C1, PS 1A; PM 1.3.1; Project #5305-21220-004-00D).

Increasing vitamin A content in maize by manipulating plant metabolism. Vitamin A deficiency results in progressive blindness, increased infant mortality, and depressed immunological responses. ARS scientists in Ithaca, New York, and university collaborators genetically mapped and identified metabolic pathway genes in maize germplasm for dramatically increasing pro-vitamin A levels in the grain. Maize breeders in Africa applied DNA markers and selection for grain color (β -carotene) to enhance pro-vitamin A levels by conventional breeding. New hybrid maize varieties containing more Vitamin A in their kernels were released in Africa in 2012 and 2013. (NP 301, C 1; PS 1B; PM 1.3.1; Project #1907-21000-029-00D)

Development and release of wheats with novel end-use properties. ARS scientists at Lincoln, Nebraska, developed wheat with altered grain quality properties in-demand by the food processing industry. Developed materials include the cultivar ‘Mattern’ and an extensive array of common and durum wheat breeding lines with waxy (amylose-free) starch. Additionally, ARS scientists identified a gene affecting wheat grain polyphenol oxidase levels. Low levels of polyphenol oxidase are required in newly released hard white wheat cultivars; the previously unidentified gene was discovered in Australian wheats developed in the 1930’s, and maintained by the USDA-ARS National Small Grains Collection in Aberdeen, Idaho. It effectively eliminates grain polyphenol oxidase in common wheat. Thus, discoloration in fresh and frozen products developed from hard white winter wheats can be avoided, and U.S. markets potentially expanded. (NP301; C1; PS1A; PM 1.3.1; Project#5440-21000-029-00D)

New thornless blackberry. ARS researchers at Corvallis, Oregon, developed, submitted for patent (S.N. 13/815,074), and released 'Columbia Star,' a thornless trailing blackberry for the commercial blackberry industry. The industry standard, 'Black Diamond,' is widely grown but lacks outstanding fruit quality, and the second industry standard, 'Marion,' is thorny and soft but has excellent flavor and processing quality. 'Columbia Star' is thornless and high yielding, with superior fruit quality as a processed and fresh marketed fruit. Every plant that was available (over 40,000) this past spring was purchased, and the nurseries are working hard to meet next year's demands. (NP301, C1, PS 1A; PM 1.3.1; Project # 5358-21000-037-00D)

Disease-resistant pea germplasm and genetic markers for accelerating resistance breeding. Pea, a valuable source of dietary protein and micronutrients, also plays a key role in the small grains-legumes crop rotation in the U.S. Pacific Northwest. The fungus *Aphanomyces* causes a devastating root rot of peas worldwide, resulting in substantial economic losses. ARS researchers in Pullman, Washington, and their university and international collaborators developed and released breeding lines highly tolerant to this root rot and also identified genetic markers to enable breeders to rapidly select disease-resistant breeding stock. These new breeding tools and genetic material will accelerate the rate of incorporating resistance into pea to increase crop yield in *Aphanomyces*-affected areas worldwide. (NP 301; C 1; PS 1B; PM 1.3.1; Project# 5348-21000-026-00D).

Genetic relationships and area of origin identified for carrots. The genetic relationships and geographical origins of crop varieties and their wild relatives provide key information for guiding crop breeding, genetic research, and genetic resource conservation. Genetic marker research conducted by ARS scientists at Madison, Wisconsin, and their university colleagues identified the area of origin (Central Asia) for carrots and determined that wild carrots from the United States were most closely related to European wild carrots. Carrots are one of the most important sources of Vitamin A worldwide, and this new information about populational genetic structure can accelerate the progress of carrot breeding and improve the efficacy of carrot genetic resource management. (NP 301; C 1; PS 1B; PM 1.3.1; Project# 3655-21000-050-00D).

Three genepools for tomato identified. Tomato, one of the most important vegetable crops worldwide, is a key source of vital vitamins and micronutrients for human diets. Tomato breeding requires new sources of genetic variability to accelerate genetic improvement for higher yield and important fruit quality traits. ARS researchers in Geneva, New York, and in Albany, California, and their university cooperators analyzed variability in SNP genetic markers and fruit quality traits from the USDA-ARS National Plant Germplasm System (NPGS) genebank collection and uncovered three distinct genepools (North American, non-North American, and cherry tomato). This new knowledge of genetic structure and fruit quality can now be applied to choosing optimal sources of diverse traits for tomato breeding. (NP 301; C1; PS 1B; PM 1.3.1; Project# 1910-21000-019-00D).

New class of wheat gliadin proteins identified. Analysis of the sequenced genome and expressed genes of bread wheat and its relatives made it possible for ARS scientists at Albany, California, to identify a new class of wheat seed gliadin genes and proteins. The major seed storage proteins including gliadin are the major determinant of the quality of wheat dough processing, thus, the complete identification of the components that form the gluten polymer is important. The wheat gluten proteins are also associated with gluten-related disorders including celiac disease. The identification of these new gluten proteins is a step in developing a successful strategy to eliminate or reduce causative factors for gluten sensitivity. (NP301; C1; PS1B; PM 1.3.1; Project#5325-21000-015-00D)

DNA sequences associated with resistance to aflatoxin contamination identified. Aflatoxin contamination of corn is a serious food and feed safety problem in the southern United States and many developing countries. Corn hybrids with genetic resistance are a key component in the strategy to eliminate or reduce contamination, and molecular markers are essential to the successful development and deployment into farmers' fields of high quality corn hybrids with resistance to aflatoxin contamination. ARS scientists in Starkville, Mississippi, developed a protocol for evaluating DNA sequences from any corn germplasm line for its role in resistance to *Aspergillus flavus* infection and subsequent aflatoxin accumulation. Five genes have been found to be highly associated with resistance, and genetic markers were developed for those genes. These markers can help to rapidly and economically transfer resistance to aflatoxin contamination into agronomically desirable, but susceptible, parental inbred lines of corn. (NP301; C1; PS1B; PM 1.3.1; Project #6406-21000-011-00D).

A gene controlling pre-harvest sprouting in hard white winter wheat identified. When harvest of mature wheat is delayed by rain, pre-harvest sprouting often occurs, especially in some hard white winter wheat cultivars. Sprouted wheat is low in quality and is often suitable only for animal feed. A sprouting tolerance gene called TaPHS1 was identified on chromosome 3A by researchers from ARS and university colleagues in Manhattan, Kansas. Many white wheat cultivars carry a nonfunctional version of this gene and thus are susceptible to pre-harvest sprouting. A DNA marker developed from the gene will allow breeders to easily and accurately select future varieties that carry tolerance to pre-harvest sprouting. (NP301; C1; PS1B; PM 1.3.1; Project#5430-21000-006-00D)

DNA-based tools developed to accelerate the development of superior varieties of small grains. DNA markers along a genome are like landmarks along a highway. The markers can assist the development of new varieties by enabling selection of segments of the genome that contain desirable genes. However, small grains like wheat and barley have extremely large genomes that require numerous markers to achieve adequate density for selection of the desirable genes. ARS scientists in Fargo, North Dakota, in collaboration with other scientists in the United States and elsewhere, developed high-density DNA marker panels that enable up to 92,000 markers distributed throughout the genome to be evaluated at once. The high-density marker panels can be applied to evaluate a many lines at a low cost and will enable U.S. small grains breeders to more rapidly and efficiently develop varieties with superior performance and quality. (NP301; C1; PS1B; PM 1.3.1; Project#5442-21000-033-00D)

New tools for breeding winter wheat adapted to diverse climates. Winter wheat is grown across a vast region and makes up 70 percent of the wheat acreage in the United States. To flower normally, all winter wheat varieties require a period of cold referred to as vernalization. By analyzing a population of lines developed from a cross between winter wheat varieties from North Carolina and Georgia, ARS researchers at Raleigh, North Carolina, determined that differences in flowering were associated with the *vrn-B1* gene. The *vrn-B1* gene was the primary determinant of flowering time at field locations in Georgia and Louisiana and also in North Carolina during 2012 and 2013, when winter temperatures were warm. A newly developed molecular marker has provided a screening tool to help breeders utilize diverse winter wheat germplasm while selecting the appropriate *vrn-B1* allele for adaptation to the local environment. (NP301; C1; PS1B; PM 1.3.1; Project #6645-22000-017-00D)

Comprehensive set of expressed genes from oat seeds developed. Oat is an important food and forage crop that lags behind wheat and barley in genome resource development. In this study, ARS scientists in Saint Paul, Minnesota, developed a new genome resource for oat improvement. High throughput sequencing data was assembled into the first comprehensive transcriptome (complete set of expressed genes) of oat seeds. The assembly is high quality and contains more than 50,000 expressed genes. Transcripts for all genes for vitamin E biosynthesis were detected, as were genes involved in the synthesis of other health-promoting compounds – beta-glucans and avenanthramides, unique polyphenol compounds with antioxidant activity. Many thousand potential new molecular markers for oat were also identified. This transcriptome assembly and the accompanying molecular markers provide new tools and resources for developing oats with higher nutritional value for human health. (NP301; C1; PS1B; PM 1.3.1; Project #3640-21000-025-00D)

Next-generation sequencing of organellar genomes in cranberry. Through next-generation sequencing technology, it is now possible to sequence whole organellar genomes of understudied, economically-important crop species such as cranberry. State-of-the-art molecular methods and computer-based approaches reconstructed the cranberry mitochondrial genome. Investigations of the cranberry mitochondrial genome are now needed to determine genome size, organization, gene content, and variation within the species and for comparison with other species. This is the first mitochondrial genome sequenced for the *Vaccinium* genus and the only one available in the entire *Ericales* order of flowering plants. The published whole mitochondrial genome of cranberry will help elucidate cellular energy production and characterize genome evolution and evolutionary relationships in this and other closely related plant species. (NP301, C1, PS 1B; PM 1.3.1; Project # 3655-21220-001-00D)

First genetic map for the blackberry. Progress with breeding of blackberries, a fruit with many valuable nutritional properties, was greatly accelerated by ARS scientists from Beltsville, Maryland, and their international partners. They developed the first genetic map for the commercial blackberry, as well as a toolbox of DNA markers for selecting key target traits such as thornlessness and production of two crops per year rather than one. Blackberry breeders and geneticists worldwide are applying these markers to develop new cultivars and to better understand the genetic control of these key traits. (NP 301; C 1; PS 1B; PM 1.3.1; Project #1245-21000-185-00D)

Discovery of molecular markers and genetic mapping of new disease resistance genes in common bean. Two unique genes conferring resistance to the devastating anthracnose and angular leaf spot diseases of the common bean were discovered in the same cultivar, and a technique called co-segregation analysis determined whether these genes were located on the same chromosome, and if so, whether they were close enough to be selected together. ARS researchers at Beltsville, Maryland, in collaboration with researchers at the State University of Maringa, Brazil, determined that the genes were very close to each other on the same chromosome and were inherited together. Through bulked segregant analysis, they discovered a DNA marker located in close proximity to the two genes, and thus can be monitored with great efficiency by the DNA marker. Moreover, these two genes occurred in a resistance gene cluster, a section of the chromosome where genes for resistance to rust, and other diseases of common bean also occur. These results are now being applied in breeding programs via marker-assisted selection to develop common bean cultivars with resistance to multiple diseases, including anthracnose and angular leaf spot. (NP 301, Component 1, PS 1B)

Component 2 – Crop Genetic and Genomic Resources and Information Management

New citrus cryopreservation method developed. The current and future productivity and profitability of the multi-billion dollar U.S. citrus industry are threatened by virulent pests and disease, such as citrus greening and citrus canker. These pests and diseases also threaten the ARS citrus breeding stock and genebank collections maintained in field and screen house plantings. Until now, preserving vegetatively propagated citrus germplasm under ultra-cold (cryopreservation) genebank conditions has not been feasible. ARS researchers in Ft. Collins, Colorado, and in Riverside, California, developed a novel micrografting technique that results in high survival rates of citrus germplasm maintained by cryopreservation. Furthermore, the new cryopreservation technique eliminates several graft-transmissible viruses and viroids. This new cryopreservation technique not only provides an effective means for safeguarding invaluable citrus germplasm, but it may also serve as a new pathogen elimination method for producing disease-free citrus propagating material. (NP 301; C 2; PS 2B; PM 1.3.1; Project# 5402-21000-012-00D and 5310-21000-010-00D).

Rich native U.S. sources of plant genetic diversity identified for crop improvement and research. The wild relatives of domesticated crops contain rich sources of genetic diversity which new genomics-assisted breeding techniques can now exploit more effectively for crop improvement. But it had long been assumed that U.S. flora contains relatively few crop wild relatives. ARS scientists in Prosser, Washington, and in Beltsville, Maryland, with international research collaborators, completed an inventory of U.S. flora that contains a rich trove (more than 4,600 different types) of crop wild relatives and wild species that could be exploited directly for food, forage, medicinal, ornamental, and industrial applications. Further comparisons of these lists with the vegetation found in parks and reserves and the holdings of the U.S. National Plant Germplasm System genebanks identified more than 800 different types of these plants as priorities for protection either in nature or in genebanks. This research has furnished a blueprint for urgent action to conserve the most endangered U.S. crop wild relatives. (NP 301; C 2; PS 2B; PM 1.3.1; Project# 5348-21000-022-00D and 1245-21000-228-00D).

Massive numbers of genetic markers developed for breeding and broadening the genetic diversity of U.S. corn. Maize (corn) is the most valuable crop in the United States with a current annual farmgate value of more than \$75 billion, but the genetic base of the U.S. maize crop is very narrow, making it potentially vulnerable to emerging diseases, pests, and environmental extremes. New methods are required to identify and conserve maize diversity, expand the crop's genetic base, and contribute valuable new traits to the U.S. maize crop. ARS researchers in Ames, Iowa; Columbia, Missouri; Ithaca, New York; and Raleigh, North Carolina, and their university collaborators developed more than 500,000 single nucleotide polymorphism (SNP) genetic markers via next-generation sequencing methods and genotyped nearly all (2,700) of the inbred lines in the U.S. National Plant Germplasm System maize collection. This vast trove of genetic data helped identify misclassified samples, elucidate the genetic relationships among poorly known lines, and, combined with agronomic descriptions of those lines, identify genes that govern key maize traits. This information will be key to accelerating maize breeding that effectively incorporates valuable new genetic diversity into the U.S. maize crop. (NP 301; C 2; PS 2B; PM 1.3.1; Project# 3625-21000-053-00D).

Metabolic pathway information grows at MaizeGDB. Understanding how plant genes function helps researchers create knowledge-based strategies for crop improvement. ARS researchers in Ames, Iowa, in collaboration with ARS scientists in Columbia, Missouri; Albany, California; and Cold

Spring Harbor, New York, as well as university collaborators, expanded the Maize Genetics and Genomics Database (MaizeGDB) metabolic network resources – MaizeCyc and CornCyc – including high quality representations of agronomically important pathways from the published literature. MaizeGDB hosts approximately 136,000 visitors per year, 97,000 of which are repeat users. (NP 301; C 2; PS 2A; PM 1.3.1; Project # 3625-21000-051-00D).

Interactive mapping program for plant specimen selection. A major goal of the U.S. National Arboretum's mission is to make its collections available for research and education. To provide internet access to these important collections, the Arboretum worked with a contractor to create a mapping system that links to an existing database of plant accessions and to a new database of images and documents. This web-based system will enable researchers to identify individual plants in the collections – including herbarium specimens – and to access detailed information about them, such as where and when they were collected. Documents and images related to the accessions will be linked to them. Visitors to the website may select and map plants of their choice to create a self-guided tour for an on-site visit to the Arboretum. (NP 301, C2, PS 2B; PM 1.3.1; Project #1230-21000-143-00D).

New genetic marker for accelerating maize breeding and genetic research. To meet the growing demand for maize (corn), the pace of yield and product quality improvement must be increased. Progress in maize breeding and genetics can be accelerated by producing uniformly homozygous plants in one generation through doubled haploid technology. ARS researchers at the Maize Genetic Stock Center, Urbana, Illinois, identified a genetically-dominant color (yellow-green) marker for identifying haploid plants rapidly and inexpensively. This marker not only could make doubled haploid technology more efficient and effective but can also contribute a more comprehensive understanding of maize photosynthesis. (NP 301; C 2; PS 2B; PM 1.3.1; Project# 3611-21000-022-00D).

Improved tissue culture methods enhance economic viability for pear. Profitability per hectare is a critical determinant for the economic viability of pear in the United States. The pear industry needs efficient means for producing thousands of pear seedlings rapidly, via micropropagation, but the tissue culture medium for pear had not been optimized for a wide range of pear varieties. ARS researchers at Corvallis, Oregon, modified the mineral nutrient content of the tissue culture medium which improved the growth and propagation success for many varieties. The new tissue culture medium could enable high density pear plantings with improved profit margins. (NP 301; C 2; PS 2B; PM 1.3.1; Project# 5358-21000-038-00D).

Component 3 – Crop Biological and Molecular Processes

Genes can move among the separate genomes of flowering plant cells. Genes consist of DNA, most of which is located in the chromosomes of the nuclear genome. But in plants, small quantities of DNA are also found in the genomes of two sub-cellular compartments, mitochondria and plastids. ARS researchers in Madison, Wisconsin, and university colleagues found that DNA originally from the carrot mitochondrial genome has moved into the carrot plastid genome – the first report of DNA transfer between those genomes for any organism. These findings advance the scientific understanding of how DNA can move between separate flowering plant genomes and the possible origins of some genetic variation found both within and among different plant species. (NP 301; C 3; PS 3A; PM 1.3.1; Project #3655-21000-048-00D).

Creating new sources of soybean genes that retard cyst nematode development. Soybean cyst nematodes attack the roots of soybean plants, causing approximately \$1 to 2 billion in damage each year. These nematodes evolve into new genetic races rapidly, especially when challenged with resistant soybeans. Very few new unique sources of resistance have been found in soybean germplasm collections. To improve soybean resistance to the soybean cyst nematode and generate new genetic diversity for breeding, ARS researchers in Beltsville, Maryland, identified approximately 50 *Arabidopsis*, soybean, and nematode genes as candidates for developing partial resistance against the soybean cyst and root-knot nematodes. DNA constructs derived from these genes were incorporated (transformed) into soybean roots and challenged with either soybean cyst nematodes or root-knot nematodes. Four DNA constructs decreased the number of cysts formed by the soybean cyst nematode by 50 percent or more and decreased the number of galls formed by root-knot nematode by 60 percent or more. These DNA constructs are very valuable tools for understanding mechanisms of plant resistance to these nematodes and for developing genetically broad resistance against nematodes. (NP 301, C3, PS 3A, PM 1.3.1; Project #1275-21220-229-00D).

Orchestration of a symphony of tomato genes by the epigenome. Tomato fruit ripening is triggered by the plant hormone ethylene; however, the impact of ethylene on fruit development is restricted by unknown cues to mature fruit. ARS researchers at Ithaca, New York, demonstrated that changes in DNA cytosine methylation, a signature feature of the epigenome, are dynamic during fruit development and ripening, and are specifically associated with the binding of key regulatory proteins that promote ripening. This finding opens an exciting new frontier for the identification of novel sources of variation for crop improvement at the epigenome level rather than DNA sequence level. (NP 301; C 3; PS 3A; PM 1.3.1; Project #1907-21000-033-00D).